

Combating antibiotic resistance in bacteria by combination drug therapy

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Imagine a physically disabled patient with a nosocomial septic arthritis due to *Staphylococcus aureus*. The chances of cure are slim because the bacterial infection is insensitive to (single) antibiotic due to mutations in the mobile genetic elements (transposons) harboured by the bacteria. The transposon DNA might code for: (a) 'efflux' pumps that eject the antibiotics from cells or (b) the genes might give rise to enzymes that degrade the antibiotics, or (c) genes that chemically alter and inactivate the drugs. Will a combination of some available antibiotics be effective against the resistant mutant than the normal (wild type) bacteria?

The results of a research carried out by the system biologist, Roy Kishony and his graduate students at Harvard Medical School bear on this problem. They used the model organism *Escherichia coli* to predict that some available antibiotics interact in different combinations to affect the growth of the resistant and wild type (normal strain)¹. The effect of drug combinations is classified as: (1) synergistic, when treatment effect is larger; (2) additive, when the effect is equal to; (3) antagonistic, when the effect is smaller than one of the drugs itself, or (4) suppressive, when the effect is weaker than that of one of the drugs alone (Figure 1).

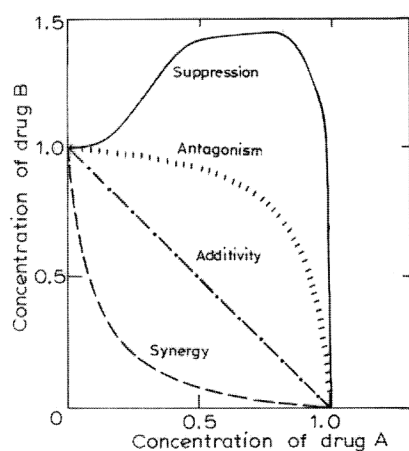


Figure 1. Diagram of types of effects of a combination of two antibiotics on growth rate of bacteria.

The measurements of individual growth rates of the sensitive and resistant strains in response to combination of two antibiotics showed that the response curve is not symmetric: there is a region of drug combination that actually selects against the resistant mutant rather than the wild type (Figure 2). When a bacterium mutant becomes resistant to a particular antibiotic, apparently additional changes occur in the resistant variant and its response to combination of two drugs shows difference at particular concentrations of the drugs in which the resistant bacteria are inhibited whereas the wild type bacteria, despite being more sensitive to the individual drugs, outcompete the resistant mutant bacteria.

The antibiotic doxycycline (DOX) inhibits protein synthesis and is used to treat a variety of bacterial infections. The authors studied the effects of this antibiotic in combination with erythromycin or ciprofloxacin on the selection of resistant and sensitive test bacterium in a growth medium. To allow comparisons of competitive selection, the DOX-sensitive and DOX-resistant types had been tagged by differential labelling using yellow or green

fluorescing proteins respectively. Their individual growth rates were monitored by luminescence using a FACS (fluorescence activated cell sorter). Furthermore, the growth rates were measured as a function of two-dimensional drug concentrations. The results showed synergy with DOX and erythromycin, and suppression with DOX and ciprofloxacin. As expected, the DOX-resistant mutant outcompeted the wild type under DOX treatment and when DOX was given with erythromycin. Remarkably, in the presence of ciprofloxacin, DOX generated selection against its own resistant mutant allele! For example, when $0.1 \mu\text{g ml}^{-1}$ DOX was added to 7.5 ng ml^{-1} ciprofloxacin. The authors emphasize that 'our work is limited to sublethal drug concentrations, in a controlled environment *in vitro* and that any possible therapeutic implications from these findings are beyond its scope'. However, these findings would lead to research into 'new treatment strategies employing antimicrobial combinations with improved selection against resistance'.

The antibiotic interactions that select against resistance may offer a powerful method to leverage the efficacy of therapeutic agents. This paper raises important issues. Is our present concept of resistance falsified? Does the origin of resistance in bacterial cells involve epigenetic changes in addition to primary change in a single gene? How does combination drug therapy tip the balance against resistance, i.e. the sensitive bacteria in population outcompeting the resistance bacteria that evolved under applied selective pressures? Are the principles in selection of bacteria also applicable to normal and cancerous human cells in tumours?

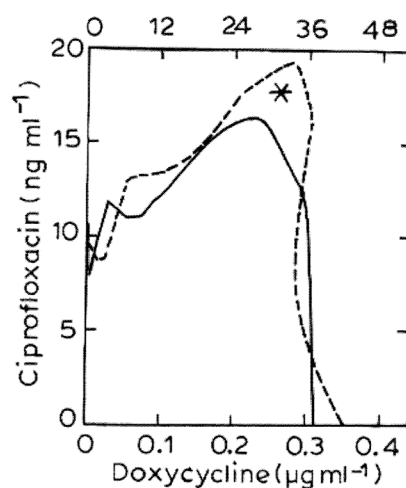


Figure 2. Growth rates as a function of combination treatments of doxycycline-ciprofloxacin. Note that in the region marked by asterisk, the sensitive bacteria (dashed line) outcompete the resistant mutant (black line).

1. Chait, R., Craney, A. and Kishony, R., *Nature*, 2007, **446**, 668–671.

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